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Resistance to myocardial infarction induced by heat stress and the effect of ATP-sensitive potassium channel blockade in the rat isolated heart

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1 Heat stress (HS) is known to protect against myocardial ischaemia-reperfusion injury by improving mechanical dysfunction and decreasing necrosis. However, the mechanisms responsible for this form of cardioprotection remain to be elucidated. ATP-sensitive potassium (K_{ATP}) channels have been shown to be involved in the delayed phase of protection following ischaemic preconditioning, a phenomenon closely resembling the HS-induced cardioprotection. The aim of this study was thus to investigate the role of K_{ATP} channels in HS-induced protection of the isolated rat heart.

2 Twenty four hours after whole body heat stress (at 42°C for 15 min) or sham anaesthesia, isolated perfused hearts were subjected to a 15 min stabilization period followed by a 15 min infusion of either 10 μM glibenclamide (Glib), 100 μM sodium 5-hydroxydecanoate (5HD) or vehicle (0.04% DMSO). Regional ischaemia (35 min) and reperfusion (120 min) were then performed.

3 Prior heat stress significantly reduced infarct-to-risk ratio (from 42.4±2.4% to 19.4±2.9, $P<0.001$). This resistance to myocardial infarction was abolished in both Glib-treated (40.1±1.8% vs 42.3±1.8%) and 5HD-treated (41.2±1.8% vs 41.8±1.2%) groups.

4 The results of this study suggest that K_{ATP} channel activation contributes to the cytoprotective response induced by heat stress.

Keywords: Heat stress; infarct size; K_{ATP} channel; glibenclamide; sodium 5-hydroxydecanoate

Introduction

Heat stress (HS) is known to induce synthesis of heat stress protein (HSPs). There is evidence to suggest that HSPs play an important role in the ability of a cell to survive noxious stresses such as a myocardial ischaemia-reperfusion sequence (Currie *et al*., 1988; Donnelly *et al*., 1992). In particular, a direct correlation between the amount of HSP72 induced and the degree of myocardial protection has been observed in the rat (Hutter *et al*., 1994) and in the rabbit (Marber *et al*., 1994). This cardioprotection, induced 24 to 48 h following heat shock, resembles that observed during the second phase of protection following ischaemic preconditioning (Marber *et al*., 1993; Yellon & Baxter, 1995). Hence, mediators under investigation for their role in ischaemic preconditioning may therefore provide a potential mechanism for heat stress-induced protection (Parratt & Szekeres, 1995; Richard *et al*., 1996). In the rat (Qian *et al*., 1996; Schultz *et al*., 1997) and in the rabbit (Parratt & Kane, 1994), some forms of cardioprotection following acute ischaemic preconditioning are known to be associated with ATP-sensitive potassium (K_{ATP}) channel opening.

Therefore, in this study we investigated the role of K_{ATP} channels in the development of HS-induced delayed resistance to myocardial infarction. The selective blockers of K_{ATP} channels, glibenclamide and sodium 5-hydroxydecanoate, were used in our model of heat stress-induced cardioprotection in the rat isolated heart (Joyeux *et al*., 1998).

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Methods

This study was conducted in two parts. The animals were first submitted to either heat stress (HS groups) or sham anaesthesia (Sham groups). Subsequently, all rats recovered for 24 h. In the second part, an ischaemia (35 min)-reperfusion (120 min) protocol was performed in isolated hearts. At the beginning of this procedure, either 10 μM glibenclamide (Glib) (Grover *et al*., 1993), 100 μM sodium 5-hydroxydecanoate (Grover *et al*., 1995) or vehicle (0.04% DMSO) were added to the perfusion medium.

Experimental treatment groups

Six experimental groups were studied: Sham + V ($n=7$) and HS + V ($n=7$) — hearts from sham-anaesthetised and heat-stressed rats, respectively, perfused with vehicle, Sham + Glib ($n=6$) and HS + Glib ($n=6$) — hearts from sham-anaesthetized and heat-stressed rats, respectively, perfused with 10 μM glibenclamide, Sham + 5HD ($n=7$) and HS + 5HD ($n=7$) — hearts from sham-anaesthetized and heat-stressed rat, respectively, perfused with 100 μM sodium 5-hydroxydecanoate.

Heat stress protocol

Heat stress was achieved by placing rats, lightly anaesthetized with pentobarbitone sodium (25 mg kg^{-1}, i.p.), in an environmental chamber under an infrared light. The body temperature, recorded with a rectal probe, was increased to 42±0.2°C for 15 min. Sham rats were anaesthetized only. All animals were allowed to recover for 24 h.

Ischaemia-reperfusion protocol

Twenty-four hours after heat stress or sham anaesthesia, rats were anaesthetized with 50 mg kg^{-1}, i.p., pentobarbitone

sodium and treated with heparin (1000 u kg^{-1}, i.p.). The heart was rapidly excised and immediately immersed in 4°C Krebs-Henseleit buffer solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.2 and glucose 11.0). The aortic stump was then cannulated and the heart perfused retrogradely by use of the Langendorff technique at a constant pressure (102 cmH_2O) with oxygenated Krebs-Henseleit buffer. A water-filled balloon, coupled to a pressure transducer (Statham), was inserted into the left ventricular cavity via the left atrium for pressure recordings. Left ventricular end-diastolic pressure (LVEDP) was adjusted between 8–12 mmHg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and maintained constant close to 37°C. For temporary occlusion of the left coronary artery (LCA), a 3/0 silk suture (Mersilk W546, Ethicon) was placed around the artery a few millimeters distal to the aortic root. After 15 min of stabilization, either 10 μM Glib, 100 μM 5HD or vehicle were added for 15 min to the perfusion solution. Following this treatment, regional ischaemia was induced by tightening the snare around the LCA for 35 min. Thereafter the heart was reperfused for 120 min. Coronary flow (CF) was measured throughout the ischaemia-reperfusion procedure, by collecting the effluent. Heart rate (HR) and left ventricular developed pressure (LVDP = difference between left ventricular systolic pressure and LVEDP) were continuously recorded on a polygraph (Windograph, Gould Instrument). At the end of the reperfusion period, the coronary artery ligature was retied and unisperse blue dye was slowly infused through the aorta to delineate the myocardial risk zone. After removal of the right ventricle and connective tissues, the heart was frozen at −18°C for 1 h and then sectioned into 2 mm transverse sections from apex to base (6–7 slices/heart). Following defrosting, the slices were incubated at 37°C with 1% w/v triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4) for 10–20 min and fixed in 10% v/v formaldehyde solution to distinguish stained viable tissue and unstained necrotic tissue. Left ventricular infarct zone (I) was determined by a computerized planimetric technique (Minichromax; Biolab) and expressed as a percentage of the risk zone (R) and the left ventricle (LV) (Speechly-Dick *et al*., 1994).

Materials

Glibenclamide (Glib) and sodium 5-hydroxydecanoate (5HD) were obtained from Sigma Chemical (France); unisperse blue dye was from Ciba-Geigy (France); 2,3,5-triphenyltetrazolium chloride was from Sigma (France). All other reagents were of analytical reagent quality.

Male Wistar rats (280–340 g) were used for these studies. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication n° 85-23, revised 1985).

Statistical analysis

All data are presented as mean ± s.e.mean. Comparisons in CF, HR and LVDP were determined by repeated measures ANOVA with *post-hoc* multiple comparisons Tukey tests. Infarct size was analysed by a one-way ANOVA. P values $\leqslant 0.05$ were considered significant.

Exclusion criteria

Only hearts with CF within 8–15 ml min^{-1} and LVDP > 70 mmHg at the end of the stabilization period were included in this study. The efficiency of coronary occlusion was indicated by a CF diminution > 30%. Hearts which developed ventricular fibrillation during ischaemia-reperfusion and which could not return spontaneously to normal sinus rhythm within 2 min were excluded. Moreover, the risk zone determined at the end of the ischaemia-reperfusion procedure had to represent 40–60% of the LV. Three hearts were excluded because they did not conform with these pre-determined criteria.

Results

Haemodynamic data

Table 1 summarizes CF, HR, and LVDP data recorded in the six experimental groups during the stabilization period and the

Table 1 Haemodynamic data for the six experimental groups

				Ischaemia			*Reperfusion*		
	Group	*Stabilization*	*Treatment*	*5 min*	*34 min*	*15 min*	*30 min*	*60 min*	*120 min*
CF	Sham + V	11.1 ± 0.6	11.2 ± 0.7	5.9 ± 0.3	5.8 ± 0.6	8.6 ± 0.7	7.0 ± 0.7	6.0 ± 0.6	5.4 ± 0.8
(ml min^{-1})	HS + V	11.0 ± 0.5	10.9 ± 0.4	6.1 ± 0.4	4.6 ± 0.4	7.8 ± 0.6	7.1 ± 0.6	5.9 ± 0.7	4.3 ± 0.8
	Sham + Glib	10.2 ± 0.6	8.4 ± 0.5	4.1 ± 0.3	5.1 ± 0.5	8.1 ± 0.5	6.7 ± 0.6	5.8 ± 0.7	4.2 ± 0.4
	HS + Glib	9.9 ± 0.4	8.9 ± 0.5	4.1 ± 0.3	4.1 ± 0.5	8.3 ± 0.5	7.4 ± 0.6	5.9 ± 0.6	4.5 ± 0.5
	Sham + 5HD	10.7 ± 0.6	10.5 ± 0.5	4.9 ± 0.5	4.8 ± 0.6	9.0 ± 0.6	7.9 ± 0.4	6.9 ± 0.5	5.7 ± 0.5
	HS + 5HD	10.3 ± 0.4	9.4 ± 0.5	4.0 ± 0.2	4.8 ± 0.4	8.3 ± 0.4	8.0 ± 0.4	6.8 ± 0.4	4.2 ± 0.4
HR	Sham + V	337 ± 2	344 ± 4	300 ± 13	302 ± 10	301 ± 10	304 ± 7	302 ± 16	306 ± 18
(beats min^{-1})	HS + V	332 ± 3	329 ± 4	300 ± 7	290 ± 10	304 ± 8	309 ± 11	301 ± 11	288 ± 16
	Sham + Glib	301 ± 8	285 ± 8	278 ± 10	285 ± 10	288 ± 10	280 ± 8	282 ± 7	272 ± 6
	HS + Glib	306 ± 7	289 ± 9	295 ± 9	286 ± 7	278 ± 10	272 ± 10	272 ± 13	270 ± 15
	Sham + 5HD	314 ± 5	303 ± 7	299 ± 7	284 ± 12	280 ± 11	287 ± 9	296 ± 8	292 ± 13
	HS + 5HD	305 ± 7	295 ± 4	285 ± 5	300 ± 13	278 ± 5	275 ± 7	272 ± 6	276 ± 9
LVDP	Sham + V	91 ± 7	87 ± 6	28 ± 3	66 ± 7	83 ± 6	75 ± 5	58 ± 9	49 ± 5
(mmHg)	HS + V	100 ± 6	106 ± 6	39 ± 4	59 ± 8	93 ± 6	82 ± 7	68 ± 10	55 ± 9
	Sham + Glib	106 ± 6	93 ± 6	29 ± 3	71 ± 6	92 ± 8	85 ± 7	70 ± 10	59 ± 6
	HS + Glib	93 ± 7	90 ± 6	29 ± 6	65 ± 4	92 ± 6	86 ± 5	70 ± 9	54 ± 6
	Sham + 5HD	92 ± 4	93 ± 4	25 ± 4	60 ± 4	96 ± 4	84 ± 5	68 ± 10	51 ± 6
	HS + 5HD	95 ± 5	89 ± 3	24 ± 2	62 ± 3	90 ± 4	74 ± 6	63 ± 8	57 ± 6

CF — coronary flow; HR — heart rate; LVDP — left ventricular developed pressure. Sham + V ($n = 6$) — sham-anaesthetized and vehicle-perfused; Sham + Glib ($n = 7$) — sham-anaesthetized and glibenclamide-perfused, Sham + 5HD ($n = 6$) — sham-anaesthetized and sodium 5-hydroxydecanoate-perfused. HS + V ($n = 6$), HS + Glib ($n = 7$), HS + 5HD ($n = 6$) — heat-stressed and similarly perfused. Data are mean ± s.e.mean.

Table 2 Risk (R) and infarct (I) sizes expressed as a percentage of the left ventricle (LV)

	Group					
	Sham + V	*HS + V*	*Sham + Glib*	*HS + Glib*	*Sham + 5HD*	*HS + 5HD*
n	6	6	7	7	6	6
R/LV (%)	51.8 ± 2.8	55.9 ± 3.4	52.7 ± 1.0	52.8 ± 0.9	50.9 ± 1.1	50.1 ± 1.2
I/LV (%)	27.0 ± 2.0	12.1 ± 1.9*	22.3 ± 1.1	21.1 ± 1.1	21.9 ± 0.9	21.5 ± 0.8
I/R (%)	42.4 ± 2.4	19.4 ± 2.9*	42.3 ± 1.8	40.1 ± 1.8	41.8 ± 1.2	41.2 ± 1.8

HS = heat-stressed; Sham = sham-anaesthetized; Glib = glibenclamide-treated; 5HD = sodium 5-hydroxydecanoate-treated and V = vehicle-treated. Data are mean ± s.e.mean. *$P < 0.001$ vs all the other groups.

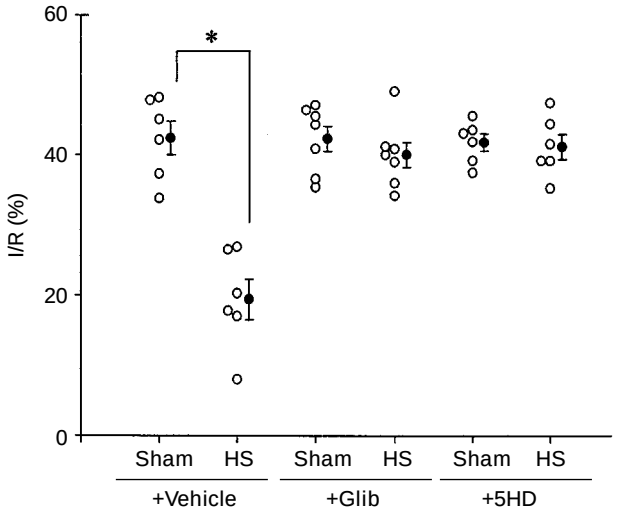

Figure 1 Percentage infarction of the risk zone in rat isolated hearts subjected to a 15 min infusion of either 10 μM glibenclamide (Glib) or 100 μM sodium 5-hydroxydecanoate (5HD) or vehicle (0.04% DMSO) followed by 35 min LCA occlusion and 120 min reperfusion. *$P < 0.001$.

ischaemia-reperfusion protocol. Twenty four hours after heat stress or sham anaesthesia, there was no statistically significant difference in haemodynamic performance at any time point between the six groups.

Infarct data

Figure 1 presents infarct size data expressed as a percentage of the risk zone (I/R) for the six experimental groups. Heat stress significantly reduced infarct size from 42.4 ± 2.4% in Sham + V to 19.4 ± 2.9% in HS + V ($P < 0.001$, one-way ANOVA). This resistance to myocardial infarction induced by heat stress was abolished by both Glib (I/R: 40.1 ± 1.8% in HS + Glib vs 42.3 ± 1.8% in Sham + Glib) and 5HD (I/R: 41.2 ± 1.8% in HS + 5HD vs 41.8 ± 1.2% in Sham + 5HD) treatment. Similar results were observed with the infarct-to-left ventricle ratio (I/LV), (Table 2). Myocardial risk size expressed as the percentage of the left ventricle (R/LV) was similar for all groups (Table 2). Differences in infarct size, therefore, did not result from variability in the risk zone.

Discussion

This study provides the first results implicating the activation of K_{ATP} channels in heat stress-induced delayed cardioprotection in the rat. In our *in vitro* rat model of myocardial infarction, heat stress significantly reduced infarct size 24 h after whole body hyperthermia. This is in agreement with data from previous studies (Donnelly *et al*., 1992; Marber *et al*., 1993; Joyeux *et al*., 1998).

This cardioprotection was abolished by K_{ATP} channel blockade with two structurally different K_{ATP} channel blockers, glibenclamide and 5-hydroxydecanoate. Unlike glibenclamide, 5-hydroxydecanoate does not possess sulphonylurea receptor activity and remains effective during ischaemia (Grover *et al*., 1995). Therefore, the delayed cytoprotection conferred by heat stress in the rat isolated heart seems to be mediated via K_{ATP} channels rather than sulphonylurea receptors. This is in accordance with a recent *in vivo* study in the rabbit (Pell *et al*., 1997).

Although the K_{ATP} channels have been demonstrated to be involved in acute ischaemic preconditioning in most species (for a review see Gross, 1995), controversy still exists as to the role of these channels as a mediator of ischaemic preconditioning in the rat heart (Qian *et al*., 1996; Schultz *et al*., 1997). Indeed, it has been observed that both glibenclamide and 5-hydroxydecanoate (at the same doses used in this study) did not block the cardioprotective effect of preconditioning (Grover *et al*., 1993; 1995).

We have previously shown in similar experimental conditions that HSP72 are induced and HSP27 are increased 24 h after heat exposure (Joyeux *et al*., 1998). A direct correlation between the amount of HSPs induced and the degree of myocardial protection has been observed by Hutter *et al*. (1994). HSPs could act direct or indirectly on K_{ATP} channels causing activation.

Calderwood and his co-workers (1988) have demonstrated that heat shock induces an increase in intracellular 1,4,5-inositol triphosphate release. This effect is antagonized by phospholipase C inhibition, suggesting that activation of phospholipase C is involved in the heat stress response and induces 1,4,5-inositol triphosphate and diacylglycerol release. We have previously shown that the activation of protein kinase C is involved in the heat stress-induced cardioprotection (Joyeux *et al*., 1998). We can, thus, presume that protein kinase C activation and K_{ATP} channel opening could occur subsequently to the activation of phospholipase C and diacylglycerol release, providing one hypothetical transduction pathway for the heat stress-induced cardioprotection.

In summary, our results show that the K_{ATP} channel opening appears to play a role in resistance to myocardial infarction of the rat isolated heart induced by heat stress. Indeed, the K_{ATP} channel blockers glibenclamide and 5-hydroxydecanoate both abolished this cardioprotection. Further investigations are required to elucidate the precise nature of K_{ATP} channel involvement in heat stress-induced myocardial protection and the possible interaction with HSPs.

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